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Seroimmunity to poliomyelitis in Sweden after the use of inactivated poliovirus vaccine for 10 years

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This study was undertaken in 1968 in collaboration with the World Health Organization as part of a co-operative evaluation of vaccination programmes. The situation in Sweden was of particular interest as only inactivated vaccines had been used for immunization against poliomyelitis. The WHO programme includes evaluation of both seroimmunity and resistance to poliovirus infection but the present report concerns only the serological studies.

About 3 000 people, selected on a statistical basis as being a representative sample of the Swedish population, were sent questionnaires concerning their vaccinations against poliomyelitis. Answers were returned by 90 % of the sample population and blood samples were collected from 2 294 persons. More than 95 % of subjects under 30 years of age had received 2 or more injections, but the proportion of vaccinated individuals decreased slightly among people over 30 years of age. In the oldest age group questioned (60–70 years) only 20 % had been vaccinated. Antibodies to the 3 types of poliovirus were present in more than 95 % of the sera in all age groups except two. Samples seronegative to one or more types of virus were found in about 15 % of people in the oldest age group and among children vaccinated during the first years of poliovirus vaccination (1957–61).

Before vaccination against poliomyelitis was available, Sweden had the highest rate of paralytic poliomyelitis in the world (Böttiger et al., 1966): furthermore, the occurrence of epidemics of the disease was first observed in Sweden. During the decade preceding the introduction of vaccination more than half of those afflicted were older than 15 years of age. All these facts indicate that the Swedish people at that time probably had the lowest natural immunity to poliomyelitis in the world. This premise and the fact that the country has so far employed only inactivated vaccine make it of special interest for immunological studies.

Since vaccination against poliomyelitis was introduced in 1957, no vaccine-associated cases of the disease have been reported and, since 1967, no case of paralytic poliomyelitis has been recorded. The numbers of poliomyelitis cases, and of new vaccinations since 1950, are shown in Table 1 together with details of virus isolations from faeces in the same period. By 1968 the total number vaccinated was 5 562 300 out of a total population of approximately 7.9 million.

MATERIALS AND METHODS

Plan of the study

A total of 3 000 persons were selected for investigation, the selection being done by the national central statistical bureau in such a way that the sample could be considered statistically representative of the population of Sweden. The people were selected from 26 districts and these were divided into 3 strata, i.e., cities, towns–villages, and rural areas.

In order to make the numbers of subjects per age group in this study comparable with those in a similar one carried out in Czechoslovakia, the younger age groups were intentionally made larger. Children born in 1957, 1959, and 1961 were excluded.

Detailed questionnaires concerning vaccination history were distributed in 1968 to the selected persons. The participants were asked to check their vaccination histories with the records of infant welfare clinics, school nurses, and health cards, etc.

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Table 1. Poliomyelitis is Sweden: epidemiology and vaccination programme, 1950–68

Year	No. of cases of poliomyelitis		No. of positive virus isolations from faeces	Type of virus isolated	Availability of vaccination (year of birth)	No. of newly vaccinated persons
	With paralysis	Without paralysis				
1950	1 164	540				
1951	339	212				
1952	363	129				
1953	3 029	2 061	454	1, 2, 3		
1954	544	455	54	1, 2, 3		
1955	325	161	160	1, 2, 3		
1956	248	301	67	1, 2, 3		
1957	148	102	81	1, 2, 3	1946–53	717 000
1958	155	37	131	1, 2, 3	1943–54	358 000
1959	42	14	81	1, 2, 3	1933–57	1 039 000
1960	15	3	16	1, 2	1920–59	489 000
1961	64	60	135	1	1910–60	1 093 000
1962	6	8	15	1, 3	1910–61	641 000
1963	0	0	0	2	1910–62	227 000
1964	0	1	1	2	1910–63	159 000
1965	1	1	2	1	all	216 000
1966	2	2	4	1	all	293 800
1967	0	0	1	1	all	190 000
1968	0	0	0		all	139 500

Subsequently, blood samples were collected by venous puncture from the participants and these were tested for poliovirus antibodies.

Measurement of antibody titres

The serum antibody titres to the three types of poliovirus were determined by the immuno-inactivation method described previously (Böttiger et al., 1969). The virus neutralizing capacity of the serum was tested in 4-fold dilution steps from 1 : 4 to 1 : 16 384. Quantities of 0.18 ml of serum and 0.02 ml of virus, containing about 4 $\log_{10}$ $TCID_{50}$ per ml, were mixed and incubated in a water-bath at 37°C for 6 h and then at +4°C overnight. The following morning the mixture was diluted 1 : 5 and 0.05 ml of it was inoculated into each of 3 tubes containing primary cultures of trypsinized monkey kidney tissue. The virus control was mixed with Hanks' solution and similarly treated; it was then diluted 1 : 5, 1 : 50, and 1 : 500 and 0.05 ml of each dilution was inoculated into each of 10 tubes. All the tubes were incubated on a roller device at 37°C. Viral cytopathic effects were scored usually after 7 days. The dose of virus mixed with the serum contained between 10 and 100 $TCID_{50}$. The mean concentration for the whole test series being 30 $TCID_{50}$ (1.5 $\log_{10}$) for type 1, 25 (1.4 $\log_{10}$) for type 2, and 30 (1.5 $\log_{10}$) for type 3.

A methodological study was performed to evaluate the influence of the virus dose used. Three sera were tested against the three types of poliovirus and against 4 different virus concentrations containing approximately 10, 100, 1 000, and 10 000 $TCID_{50}$ in the final inoculum. The mean difference between serum titres when the doses of virus were 1 000 and 10 000 $TCID_{50}$ was 10-fold, i.e., the serum titre was 10 times lower when tested against the higher virus dose. The corresponding difference be-

tween 100 and 1 000 $TCID_{50}$ was 5-fold and between 10 and 100 $TCID_{50}$ (the limits allowed in this study) was 3-fold.

Thus, at the virus concentration used, variations in the apparent serum titres due to variations in the virus titre were less than if higher concentrations had been used.

RESULTS

Vaccination history

Questionnaires were sent out to 3 031 persons and 2 734 replies were received. Of the persons who did not reply, 41% had not received the questionnaire, and 55% received the inquiry but did not reply. The percentage return of completed questionnaires did not vary between the cities, the villages, and the rural areas: 860 of the returned questionnaires concerned children from 2 to 5 years of age, 1 256 concerned children from 7 to 14 years of age, and 618 were from adults.

Fig. 1 shows the vaccination histories of the sample population according to age group and to type of community. The vaccination patterns were fairly similar in the three different types of area investigated. In the groups under 30 years of age, more than 90% reported having received one or more injections. In the four older age groups, born between 1898 and 1927, the frequency of vaccination decreased with age.

The vaccination procedure recommended by the Swedish Medical Board includes 2 primary injections 1 month apart, a third injection 1 year later, and a fourth 4 years later. Fig 2 gives the percentages of participants who claimed to have followed these recommendations. Persons who within the last 4-year period had received 3 injections and not yet reached the recommended time for the fourth injection are also included. Of the persons in the age group 2–19 years, 87% were vaccinated according to the official recommendations. The corresponding figure for the groups between 20 and 59 years of age was 65% and for the oldest group (60–69 years) it was only 14%.

Antibody titrations

Blood samples were taken from 2 294 individuals, this figure representing between 65% and 80% of the initially selected populations in each age group. The lowest participation rate (65%) was in the youngest age group.

Percentage of seronegative individuals. Among vaccinated participants a larger number of persons seronegative to type 1 was found in the age groups born in the years 1948–53 and 1954–58 (14% and 11%, respectively) than in the other age groups. A maximum of 9% of individuals seronegative to type 3 was found in persons born in the years 1948–53.

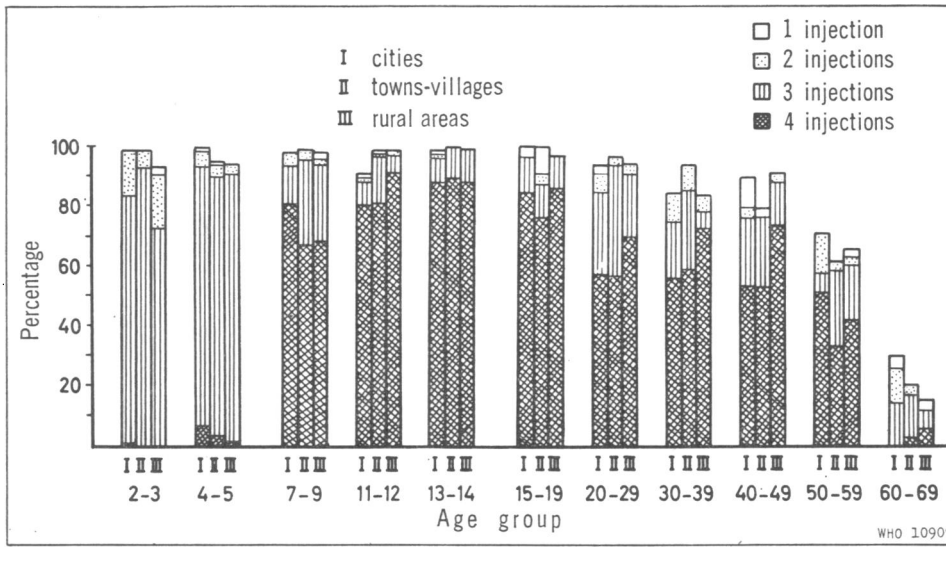

Fig. 1. Distribution of number of injections received in relation to age and to type of community.

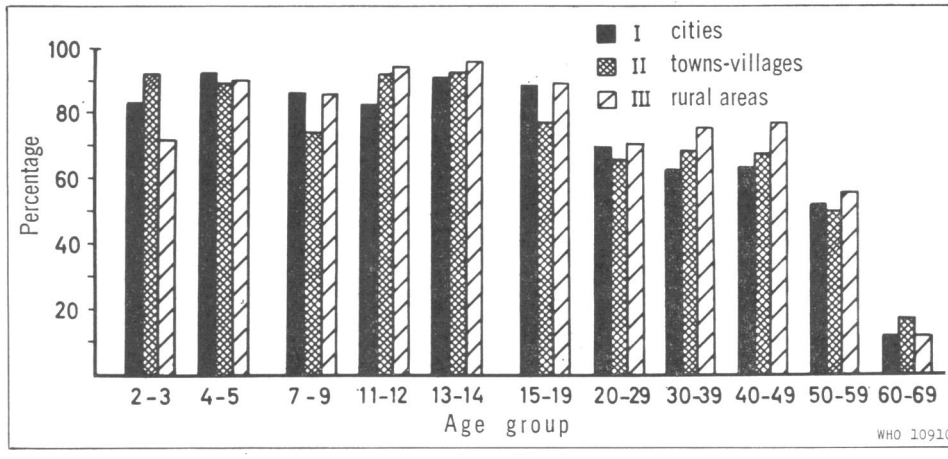

Fig. 2. Percentage of participants vaccinated according to the recommendations of the Swedish Medical Board.

In the other age groups the proportions of seronegative sera were very small: only 5 vaccinated people were seronegative to type 2 (Table 2). One adult (i.e., born before 1948) and 16 children (born in 1948 or later) were found to be triple negative: all of them were unvaccinated.

The number of unvaccinated persons in each group was relatively small. Of the 2 294 persons investigated only 433 (19%) claimed that they had not received any vaccination against poliomyelitis. Although the numbers of persons in the groups born after 1928 are small, the values in Table 2 are given as percentages to facilitate a comparison with the vaccinated group. In spite of the small numbers, an increase in the percentage of seronegative individuals in the younger age groups is apparent. No differences were observed between the different types of community.

Table 2. Percentage of persons lacking antibodies to one or more types of poliovirus in relation to vaccination history and age *

Groups according to year of birth	Vaccinated				Unvaccinated			
	No.	% seronegative			No.	% seronegative		
		Type 1	Type 2	Type 3		Type 1	Type 2	Type 3
Adults								
1898–1907	18	—	—	6	65	8	14	14
1908–1917	64	2	—	—	27	11	26	33
1918–1927	77	1	—	—	10	40	10	30
1928–1937	73	8	3	1	7	29	14	43
1938–1947	87	2	—	2	5	40	20	40
Children								
1948–1953	265	14	—	9	—	—	—	—
1954–1958	740	11	0.1	0.4	8	88	100	100
1960–1965	823	4	0.1	2	22	78	64	64

* The vaccination history was unknown in 3 of the 2 294 persons from whom blood samples were taken.

Table 3. Percentages of persons, grouped according to vaccination history, who lacked antibodies to one or more types of poliovirus

Vaccination history	Children	Adults
unvaccinated	77	43
2–3 injections 1957–60 [a]	23	4
4 injections 1957–60	15	
2–3 injections 1961–66 [a]	5	
4 injections 1961–66	1	
1–2 injections 1967	26	
date unknown	14	

[a] Only 1 or 2 persons had had only 2 injections.

The vaccination histories of the seronegative persons were classified according to the number of injections received and according to the date of the injections, and Table 3 shows the proportions of individuals with the same vaccination patterns that lacked antibodies to one or more types of poliovirus. Among the unvaccinated adults, 45% lacked antibodies to one or more types of poliovirus while among vaccinated adults only 4% (12 persons) were found to lack antibodies. Among the vaccinated children, the greatest number of seronegatives (with exception of the youngest who had not yet received 3 injections) was found among those immunized during the years 1957–60: 15% of the children who began their series of injections during this period and who had received 4 injections were seronegative.

Mean titres in relation to age, vaccination, and community. The geometric mean titres for the three types of poliovirus in vaccinated and unvaccinated persons are illustrated in Fig. 3 (titres below 1 : 4 were counted as 0.0 $\log_{10}$). The type 2 antibody titres in vaccinated persons, in contrast to those for types 1 and 3, showed mean levels at or above 2.5 $\log_{10}$ in all age groups. The type 1 antibody titres were similarly high but were reduced to 1.9 $\log_{10}$ in the groups born between 1948 and 1958. A lower level of 1.9 $\log_{10}$ was also found in the type 3 titres of people in the group born in the years 1948–53.

The mean antibody titre of vaccinated adults born between 1898 and 1947 was, in general, about 1.0 $\log_{10}$ higher than that of unvaccinated adults. In young adults and children, the majority of whom appeared to lack natural immunity, the difference between the antibody titre of vaccinated and unvaccinated persons was considerably greater and was at least 2 $\log_{10}$.

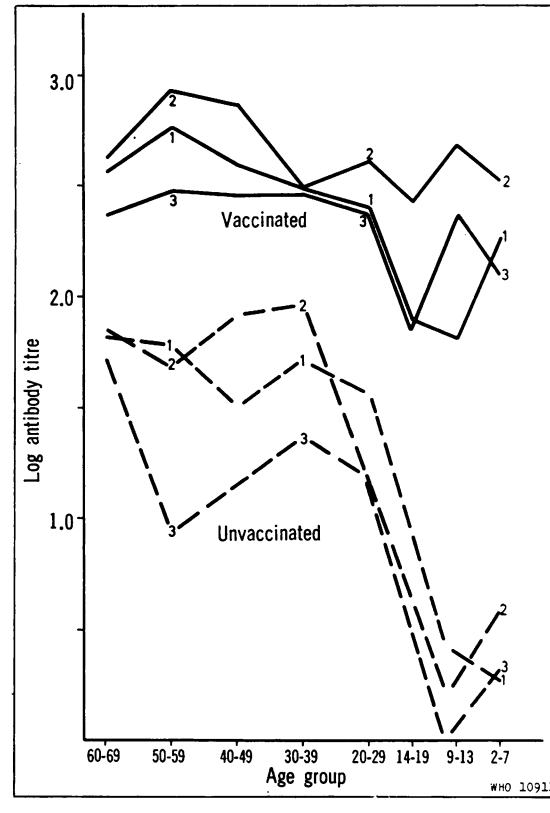

Fig. 3. Mean log antibody titres against poliovirus types 1, 2, and 3 in vaccinated and unvaccinated persons.

Mean titres in relation to vaccination history and age. The influence of the number of injections received on the antibody titres and the relation of the titres to the date of immunization was evaluated in three ways: first, by comparing the older with the younger age groups; second, by evaluating separately the titres of individuals who had received 3 or 4 injections; [1] and third, by comparing antibody titres according to the date of the first injection. Since more than 95% of the persons had received their 3rd or 4th injections within the intervals recommended by the medical board, it was considered sufficient to evaluate the results according to the time of the first injection. The 16% of the vaccinated sample donors who could not give even the

[1] Only a few adults had received only 3 injections and they were excluded from the later calculations.

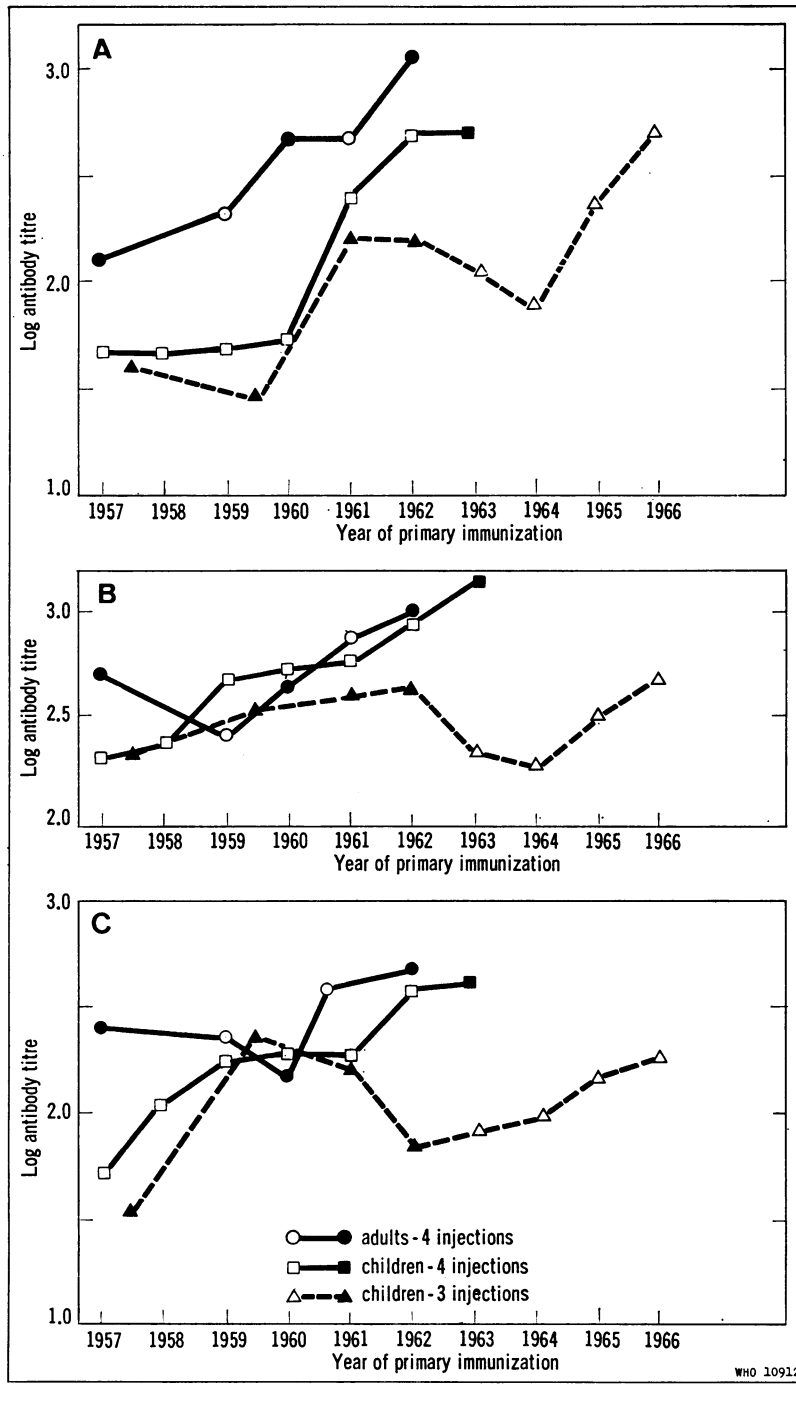

Fig. 4. Mean log antibody titres in relation to age, to year of first injection, and to number of injections: (A) type 1 antibody titres; (B) type 2 antibody titres; (C) type 3 antibody titres. The points represented with solid symbols are based on the mean of a smaller number of values (see text p. 147).

approximate date of their vaccinations were excluded from this part of the study.

An evaluation of the reliability of the vaccination data showed that 80% or more of the children of school age or younger were vaccinated within the recommended time. The corresponding figures for the adults ranged between 37% and 66%. From these findings it might be assumed that at least the data for the children were fairly correct.

Fig. 4 illustrates the antibody titres of types 1, 2, and 3 viruses, respectively. The average figures for the adults are in most cases based on the results of 12–54 determinations; those for children are based on 86–226 determinations. However, a few values are based on only 15–30 determinations: these points are marked on Fig. 4 with solid symbols.

The type 1 antibody titrations (Fig. 4A) showed that the adults who had had 4 injections had antibody titres about 3 times those of children who had had 4 injections starting in the same year. The groups of children vaccinated during the period 1957–60 showed markedly lower titres than those vaccinated later. The difference between the titres in children who had received 3 injections and those who had received 4 appeared to be at least 3-fold (0.5 $\log_{10}$) for those who started their injections in 1962 and 1963. The differences in the titre levels between age groups and between different years of primary vaccination were much less marked for type 2 antibodies (Fig. 4B) and the levels were generally high in all the vaccinated persons.

The mean titres of type 3 antibodies was also different in young people and adults but only in those vaccinated with the first vaccines, i.e., those immunized during 1957 and 1958 (Fig. 4C). This difference disappeared in those vaccinated in or after 1959. As with the titres of type 1 antibodies there was at least a 3-fold (0.5 $\log_{10}$) difference between the titres of those who had had 3 and 4 injections, respectively, when the first injections were in 1962 or 1963. For all three virus types, there were no apparent titre differences between those few participants who had their first vaccinations between 1957 and 1961 and who claimed to have received only 3 injections and those who had had 4 injections starting during the same period.

Both the type 1 and the type 2 antibody titres were slightly lower in children vaccinated in 1963 and 1964. The same phenomenon was observed in a similar pilot study performed in 1967 in the Stockholm city area on children 2–7 years of age (Böttiger, 1969).

DISCUSSION

Replies to the questionnaire

When questionnaires of this type are used it is important to consider the reliability of the information obtained. Altogether 5% of the sample population refused or neglected to return the questionnaire. It is clear that the people in this group might also be those most likely to neglect their vaccinations, a factor that might increase slightly the proportion of unvaccinated persons. Some of those who did reply might have had difficulty in remembering the date of their vaccinations: the vaccinations of younger children are recorded at the infant welfare clinics and are registered on health cards that are given to the mothers. Adults who were vaccinated were given special cards but the health officials registered only the number of people vaccinated and not their names.

The vaccination data in the replies received, however, especially those for children, were in agreement with the periods when people were offered vaccination. The data on the number of injections received are less easy to check and are less likely to be correct, especially for the individuals vaccinated a long time ago. All these factors have to be considered when the results of the antibody titres are considered in relation to vaccination history.

Even with all these reservations, it appears that the medical board has succeeded as well as could be expected in reaching all the main groups at risk (persons born before 1910 were judged to be less likely to acquire the disease) with their voluntary vaccination programme. It was to be expected that not all the participants would contribute blood samples, but the number of blood donors (84% of the persons returning the questionnaire) was regarded as satisfactory. The vaccination histories of the blood donors did not deviate statistically from those of all the persons who returned questionnaires and thus they might be regarded as a representative sample.

Antibody titres

It is not yet possible to define the antibody titre necessary to give protection against the paralytic manifestations of poliomyelitis, and indeed it might be different in different individuals. Cases of paralytic poliomyelitis reappeared in Houston in 1968 (Melnick et al., 1969) after an extensive vaccination campaign in 1962 using oral vaccine. In that case the series of vaccinations reached only about 50% of the pre-school children, however, and in 1969,

up to 50% of the children were found to be seronegative to one or more poliovirus types. In Finland, on the other hand, where as many as 50% of children, below 5 years of age were also found to be seronegative to one or more types of poliovirus, no cases of paralytic poliomyelitis have been reported (Lapinleimu, 1967). In that country, however, at least 90% of the children had been vaccinated with inactivated vaccine. It is interesting to consider whether submeasurable immunity levels can also give protection: some results from a previous study in Sweden (Böttiger & Lagercrantz, 1969) appear to support this possibility. Fourteen primary non-immune adults were given 2 doses of inactivated vaccine: 5 years later they were all found to be seronegative. Upon restimulation with one injection, 8 reacted after 14 days with an antibody rise that persisted at almost the same level for 2 months, while 6 were seropositive after 14 days but were again found to be seronegative after 2 months. It is probable that the response of the former group was similar to an anamnestic or secondary response, indicating the existence of a kind of basic immunity.

The Finnish and Swedish methods of antibody titration have been compared and were found to be equally sensitive. If protection and " herd " immunity is obtained in Finland when only 50% of the vaccinated persons show neutralizing antibodies then it appears probable that the immune levels observed in Sweden ought to be quite safe, as in none of the investigated age groups were there more than 15% that lacked demonstrable antibodies against one or more of the types of poliovirus.

The analysis of the relation between age, immunity levels, number of injections, and date of the primary immunization revealed a definite correlation between immunity status and date of immunization. All the individuals vaccinated during the 4-year period from 1957 to 1961 had markedly lower mean antibody levels to type 1 poliovirus than did those immunized later. Those whose series of injections started in 1957 also had markedly lower titres to type 3 than did people vaccinated later. These results are in agreement with the results of regular tests on the immunogenicity of the vaccines used (Böttiger & Arro, 1967). Type 1 antigenicity was considerably improved in 1961–62 and type 3 antigenicity was low in the imported vaccine used in 1957: the Swedish vaccine used from 1958 onwards, however, induced an antibody response that was 10 times higher. A course of 3 injections seems always to have given high type 2 antibody levels.

No conclusions can be drawn from the similarity of titre levels between those who received 3 or 4 injections beginning in the years 1957 to 1961. First, the group that had had 3 injections was small, and second, it is probable that a number of them had forgotten that they had had a fourth dose.

The type 2 component of the vaccine appears to be the ideal antigen. It induces high antibody levels in all persons whatever their immune status and it seems likely that these levels may be optimum values that are difficult to exceed. Such a high immunogenicity must, of course, be the goal with the type 1 and type 3 components also. Since the type 1 immunity levels are still found to be higher in vaccinated adults than in children, the optimum level has probably not yet been reached. Optimum antibody levels to type 3 poliovirus seem to have been induced during the years 1959 and 1960 when type 3 antigenicity, according to the vaccine tests, was particularly high. Adults and children vaccinated in those years thus reached similar and high mean antibody titre levels to this poliovirus type.

RÉSUMÉ

SÉRO-IMMUNITÉ À L'ÉGARD DE LA POLIOMYÉLITE, EN SUÈDE, APRÈS 10 ANS D'UTILISATION DU VACCIN ANTIPOLIOMYÉLITIQUE INACTIVÉ

Il était intéressant d'étudier les aspects actuels de l'immunité envers la poliomyélite au sein de la population suédoise. En effet, avant l'ère de la vaccination, la Suède avait le taux de poliomyélite paralytique le plus élevé du monde, indice d'une immunité naturelle particulièrement faible. La vaccination y a été introduite en 1957 et on a toujours eu recours au vaccin inactivé. En 1968, le nombre des personnes vaccinées était de 5 562 300 sur une population de 7,9 millions d'habitants.

L'enquête menée en 1968 a porté sur 3000 sujets environ choisis de façon à fournir un échantillon représentatif de la population. Ils ont été invités par questionnaire à indiquer leurs antécédents en matière de vaccination antipoliomyélitique; 2734 d'entre eux (90%) ont répondu. On a par ailleurs prélevé du sérum et recherché les anticorps antipoliomyélitiques chez 2294 personnes représentant 76% du groupe initialement sélectionné.

D'après les réponses, plus de 90% des sujets âgés de

moins de 30 ans avaient reçu au moins deux injections de vaccin inactivé. Dans les groupes d'âge supérieur, la proportion des sujets vaccinés diminuait légèrement; chez les sujets âgés de 60 à 70 ans, elle n'était plus que de 20%.

Les examens sérologiques ont montré que dans tous les groupes d'âge, sauf deux, plus de 95% des sérums contenaient des anticorps dirigés contre les trois types de poliovirus. Des sérums négatifs pour un ou plusieurs types ont été trouvés chez 15% environ des sujets du groupe d'âge le plus élevé, comptant aussi le plus faible pourcentage de sujets vaccinés, ainsi que chez les enfants vaccinés durant les premières années de la mise en pratique de la vaccination antipoliomyélitique (1957-1961).

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